DETERMINATION OF PHYTOCHEMICAL IN EXTRACTED PUNICA GRANATUM PEELS AND ITS EFFICACY OF HEALING BURN INJURIES

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ABSTRACT

Natural plants have been used as a traditional medicine all over the world. One of these natural plants is pomegranate (punica granatum). This study aimed to determine the phytochemicals from Pomegranate peels that can heal burn wounds by extracting the peels. Additionally, this study is investigating the efficiency of Pomegranate (Punica granatum) in curing the burn wounds compare to the commercial ointment. Extracting the pomegranate start with drying the peels under room temperature, followed by drying in the oven for 48 hours with the temperature of 50^{o}C, then grinding them. However, after the preparation of the ointment from the peels fine powder, four different samples of rats were burnt and treated. The result of the extract and the analysis showed that pomegranate is reach by an active constituent such as Saponin, Glycosides, Steroids and Terpenoid which play the main role in healing the burn wounds. This claimed is supported by the result of the treatment observations of the rats' samples which proof that the efficiency of using pomegranate ointment in curing the wounds is higher and faster than the commercial ointment. As conclusion the objective of this study has been achieved, and positive result has been obtained.

ABSTRAK

Tumbuhan semula jadi telah digunakan sebagai ubat tradisional di seluruh dunia. Salah satu daripada tumbuh-tumbuhan semula jadi adalah delima (Punica granatum). Kajian ini bertujuan untuk menentukan fitokimia dari kulit delima yang boleh menyembuhkan luka terbakar dengan mengekstrak kulitnya. Tambahan lagi, kajian ini menyiasat kecekapan Delima (Punica granatum) dalam menyembuhkan luka terbakar berbanding dengan ubat komersial. Prosedur untuk mengekstrak delima bermula dengan pengeringan kulit di bawah suhu bilik, diikuti dengan pengeringan dalam ketuhar selama 48 jam dengan suhu 50^{o}C, selepas itu dikisarkan. Walau bagaimanapun, selepas penyediaan ubat dari serbuk halus delima, empat sampel tikus yang berbeza telah dibakar dan dirawat. Hasil ekstrak dan analisis menunjukkan bahawa delima mengandungi konstituen aktif seperti Saponin, glikosida, Steroid dan terpenoid yang memainkan peranan utama dalam penyembuhan luka terbakar. Dakwaan ini disokong oleh hasil pemerhatian rawatan sampel tikus yang terbukti bahawa potensi ubat hasil dari ekstrak delima dalam menyembuhkan luka adalah lebih tinggi dan lebih cepat daripada ubat komersial. Sebagai kesimpulan, objektif kajian ini telah dicapai, dan keputusan yang positif telah diperolehi.

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CHAPTER I

INTRODUCTION

1.1 Background of Study

The life is filled by the different types of diseases. However, human has been searching for the solutions for those diseases till today. Natural plants have been used as a traditional medicine all over the world. The uses of the natural plants were investigated in term of its ability to cure a specific type of diseases (Nizamuddin et al., 1982; Kapoor, 1990; Barthakur and Arnold, 199, Fengshu et al., 1992, El-Mekkawyet al., 1995). Till today, the researches on the application of natural plants as medicine and their aptitude to cure the diseases are developing. Nature has been a source of medicinal agents for thousands of years and last recent years, plenty inventions and researches has been done to analyzed the hidden value inside these nature herbs. These plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The idea of developing medicines from plants used in aboriginal medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex (Heinrich and Gibbons 2001). Wounds and particularly chronic wounds are major concerns for the patient and clinician as well; chronic wounds affect a large number of patients and seriously reduce their quality of life. Balick and Cox (1996) reported that only 1–3% of medicines listed in Western pharmacopoeia are intended for use in the skin and for wounds, by comparison, at least one third of herbal remedies are for such use.

1.2 Research Background

Pomegranate (PG) or Punica granatum, is normally drink as juice in some countries. Pomegranate is one of the antique fruit that is widely consumed as fresh fruit and juice. The use of pomegranate fruit dates back to Biblical times and hearsays of its therapeutic qualities have echoed throughout the millennia (Longtin, 2003). Pomegranate, also easily acquired from traditional medicine markets, was usually used as an astringent agent, for eliminating parasites and as an antipyretic. The pharmacological functions of pomegranate include antioxidants, anti-tumors, anti-hepatotoxicity, anti-lip peroxidation and anti-bacteria properties (Alper & Acar, 2004), (Mudzhiri, 1954; Raj, 1975), (Lansky & Newman, 2007), (Khan, Afaq, Kweon, Kim, & Mukhtar, 2007; Lansky & Newman, 2007), (Kaur, Jabbar, Athar, & Alam, 2006) , (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007) , (Menezes, Cordeiro, & Viana, 2006).

Punica granatum is widely used as medicine. Tzulker et al. (2007) reported that the homogenates prepared from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril or seed sacs (fleshy or brightly colored cover of seed) juice. Wound healing involves a chain of well-orchestrated, biochemical and cellular events, leading to the growth and regeneration of wounded tissue. In coetaneous wound healing, the inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibers that increases the tensile strength. Although the rate of collagen synthesis slow

down after about three weeks, collagen cross-linking and reorganization occur for months after injury in the remodeling phase of repair (Beanes et al. 2003).

Simple extraction method of pomegranate peels has been reviewed to make sure the objective targeted is achievable. The extracts of the Pomegranate differ in composition according to the starting material and extraction method selected. These were proved with several studies done before with different by different types of method of extraction and resulted of different types of constituents.

1.3 Objectives of The Study

1- To extract and obtain the phytochemicals from Pomegranate peels that can heal burn wounds by carry out Pomegranate ointment application.

2- To investigate the efficiency of Pomegranate (Punica granatum) in curing of burn wounds compare to the commercial ointment.

1.4 Scope of Study.

This study was focusing in the pomegranate efficiency in healing the wounds through carrying the extraction and application. In addition, the application was carried out in two different phases, one is the soap and the second phase is ointment. The efficiency of pomegranate was investigated by applying the ointment on the sample rats.

CHAPTER II

LITERATURE REVIEW

A review of previous researchers and studies in the Medical Plants field will be explained in this chapter, which including Medical Plants, introduction and history of medical plants, wounds treatment procedure and types, and Pomegranate as medical Plant.

2.1 Medicinal Plants

2.1.1 Introduction

Herbal Medicine occasionally referred to as Herbalism or Botanical Medicine, is the use of herbs for their healing or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Kaur et. al, 2006). Herbalists use the leaves, flowers, stems, berries, and roots of plants to inhibit, relieve, and treat illness. From a "scientific" viewpoint, various herbal treatments are measured experimental. The actuality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine

(an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants.

Figure 2.1 Some Types of Medical Plants.

2.1.2 History of Medical Plants

In the last few years, people start to look for the last resort of healing diseases which is, nature herbs. This nature herbs or known as medicinal plants among the scientist are the main ingredients to heal up variety of sickness for those who lives at rural area. In 1993, the World Health Organization reported that 80% of the world's population rely primarily on traditional medicine and a major part of the traditional therapies involve the use of their active constituents (Thomas, 1999).

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and paregoric, a treatment for diarrhea Laudanum, a tincture of the opium poppy, was the favored tranquilizer in Victorian times. Till today, morphine-the most important alkaloid of the opium poppy-remains the standard against which the new synthetic pain relievers is measured.

Regarding the randomly use of antimicrobial drugs the microorganisms have developed resistance to many antibiotics that will lead to massive clinical problem in the treatment of infectious diseases (Davis, 1994). Meanwhile, Idose et al. in their study added to this problem, antibiotics are sometimes related with adverse effects on host which include hypersensitivity, exhaustion of beneficial gut and mucosal microorganisms and immunosuppression. Consequences from that, the need to develop alternative antimicrobial drugs for the treatment of infectious diseases becomes a priority.

Figure 2.2 Egyptian Herbal Treatment.

The use of plants as medicine is older than recorded history. As mute witness to this fact marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq. These three medicinal herbs continue to be used today. Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as a sore

throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time-honored cold and fever remedy that may once have been used much as aspirin is today.

In 2735 B.C., the Chinese emperor Shen Nong wrote an authoritative treatise on herbs that is still in use today. Shen Nong recommended the use of Ma Huang (known as ephedra in the Western world), for example, against respiratory distress. Ephedrine, extracted from ephedra, is widely used as a decongestant. You'll find it in its synthetic form, pseudoephedrine, in many allergy, sinus, and cold-relief medications produced by large pharmaceutical companies.

Figure 2.3 Chinese Herbal Treatment

The records of King Hammurabi of Babylon (c. 1800 B.C.) include instructions for using medicinal plants. Hammurabi prescribed the use of mint for digestive disorders. Modern research has confirmed that peppermint does indeed relieve nausea and vomiting by mildly anesthetizing the lining of the stomach.

The entire Middle East has a rich history of herbal healing. There are texts surviving from the ancient cultures of Mesopotamia, Egypt, and India that describe and illustrate the use of many medicinal plant products, including castor oil, linseed oil, and white poppies. In the scriptural book of Ezekiel, which dates from the sixth century B.C., we find this admonition regarding plant life: "and the fruit thereof shall be for meat, and leaf thereof for medicine." Egyptian hieroglyphs show physicians of the first and second centuries A.D. treating constipation with senna pods, and using caraway and peppermint to relieve digestive upsets.

Throughout the middle Ages, home-grown botanicals were the only medicines readily available, and for centuries, no self-respecting household would be without a carefully tended and extensively used herb garden. For the most part, herbal healing lore was passed from generation to generation by word of mouth. Mother taught daughter; the village herbalist taught a promising apprentice. By the seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. In 1649, Nicholas Culpeper wrote A Physical Directory, and a few years later produced The English Physician.

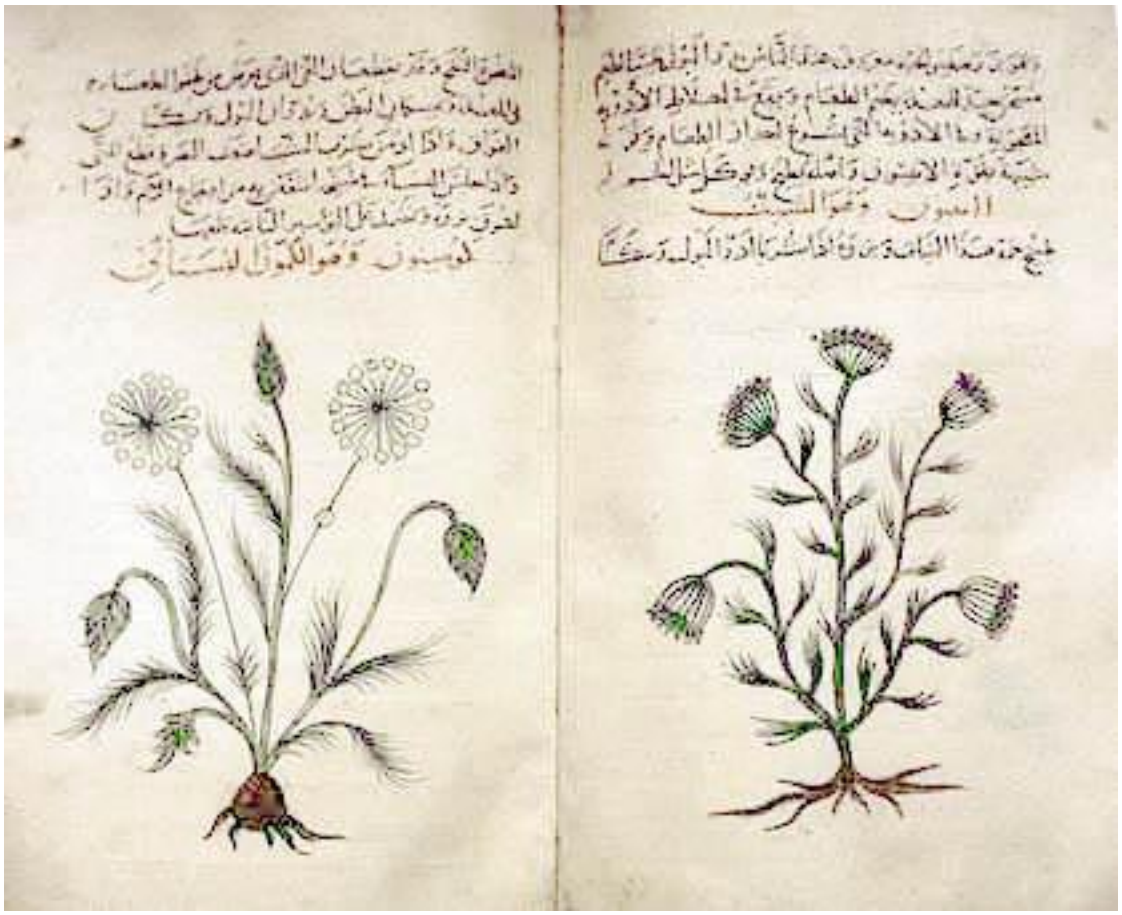

Figure 2.4 Traditional Arabic Medical Book

This respected herbal pharmacopeia was one of the first manuals that the layperson could use for health care, and it is still widely referred to and quoted today. Culpeper had studied at Cambridge University and was meant to become a great doctor, in the academic sense of the word. Instead, he chose to apprentice to an apothecary and eventually set up his own shop. He served the poor people of London and became known as their neighborhood doctor. The herbal he created was meant for the layperson.

The first U.S. Pharmacopeia was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages, and tests

of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western medicine evolved from an art to a science in the nineteenth century, information that had at one time been widely available became the domain of comparatively few. Once scientific methods were developed to extract and synthesize the active ingredients in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional, and to fall into relative obscurity.

Table 2.1 Important potential interactions between herbal preparations and conventional drugs.

Herb	Conventional drug	Potential problem
Echinacea used longer than 8 wks	Anabolic steroids, methotrexate, amiodarone, ketoconazole	Hepatotoxicity
Feverfew	Nonsteroidal anti-inflammatory drugs	Inhibition of herbal effect
Feverfew, garlic, ginseng, ginko, ginger	Warfarin sodium	Altered bleeding time
Ginseng	Phenelzine sulfate	Headache, tremulousness, manic episodes
Ginseng	Estrogens, corticosteroids	Additive effects
St John's wort	Monoamine oxidase inhibitor and serotonin reuptake inhibitor antidepressants	Mechanism of herbal effect uncertain. Insufficient evidence of safety with concomitant use—therefore not advised
St John's wort	Antiretrovirals, digoxin, theophylline, cyclosporin, oral contraceptives	Decreased clinical effect
Valerian	Barbiturates	Additive effects, excessive sedation

Table 2.1 Continued

Kyushin, licorice, plantain, uzara root, hawthorn, ginseng	Digoxin	Interference with pharmacodynamics and drug level monitoring
Evening primrose oil, borage	Anticonvulsants	Lowered seizure threshold
Shankapulshpi (Ayurvedic preparation)	Phenytoin	Reduced drug levels, inhibition of drug effect
Kava kava	Benzodiazepines	Additive sedative effects, coma
Echinacea, zinc (immunostimulants)	Immunosuppressants (such as corticosteroids, cyclosporin)	Antagonistic effects
Kelp	Thyroxine	Iodine content of herb may interfere with thyroid replacement
Licorice	Spironolactone	Antagonism of diuretic effect
Karela, ginseng	Insulin, sulfonylureas, biguanides	Altered glucose concentrations. These herbs should not be prescribed in diabetic patients

Data from Miller LG. Herbal medicinal: selected clinical considerations focusing on known or potential drug-herb interactions. *Arch Intern Med* 1998; 158:2200-2211.

The presence and concentration of a biologically active component was followed to decide the genuineness of the drug or formulation. The presence of any other component apart from the biomarkers indicates adulteration. Knowledge of these compounds and their specific analytical methods will facilitate the herbal industry in checking adulteration and thus raise its standards (Khan et. al, 2007). Punica granatum is a deciduous tree belonging to family Punicaceae. It is known for its edible fruit. During the last two decades, the drug has been subjected to extensive phytochemical, pharmacological and clinical investigations, with many interesting findings reported in various fields. Punica granatum is found to contain hydrolysable tannins as major active chemical constituents namely punicalagin, punicalin, gallic acid, ellagic

acid and ellagic acid derivative such as ellagic acid, 3, 3'-di-O-methyl, ellagic acid, 3, 3', 4'-tri-O-methyl, ellagic acid, 3'-O-methyl-3, 4-methylene; phenolic compounds such as pedunculagin, punicacortein A–D, granatin A and B, punicafolin, punigluconin, corilagin. The phytochemical constituents namely corilagin, ellagic acid, kaempferol, luteolin, myricetin, quercetin, quercimetrine, quercetin-3-O-rutinoside were previously isolated from the fruits of Punica granatum.

2.2 Wound

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue (Bennet, 1988). Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The human body is truly remarkable. Wound healing is an ability possessed by the body to repair damaged parts – this process is sometimes visible (a cut improves) or microscopic (damaged cells are replaced) but both occur on a daily basis, and are given the term: regeneration. In the animal kingdom – a starfish can rebuild a new tentacle that is cut off, an earthworm can replace much of its body that is lost, and crabs can rebuild a new claw when one is lost. There are three phases of wound healing: the inflammatory, fibroblastic, and maturation stages.

Table 2.2 Characteristics and Uses of Wound-Dressing Materials

Category	**Examples**	**Description**	**Applications**
Alginate	AlgiSite, Comfeel,	Alginate dressings are made of seaweed extract contains guluronic and	These dressings are highly absorbent and

	Curasorb, Kaltogel, Kaltostat, Sorbsan, Tegagel	mannuronic acids that provide tensile strength and calcium and sodium alginates, which confer an absorptive capacity. Some can leave fibers in the wound if they are not thoroughly irrigated. These dressings are secured with secondary coverage.	useful for wounds have copious exudate. Alginate rope is particularly useful to pack exudative wound cavities or sinus tracts.
Hydrofiber	Aquacel, Aquacel-Ag, Versiva	An absorptive textile fiber pad, hydrofiber is also available as a ribbon for packing of deep wounds. This material is covered with a secondary dressing. The hydrofiber combines with wound exudate to produce a hydrophilic gel. Aquacel-Ag contains 1.2% ionic silver that has strong antimicrobial properties against many organisms, including methicillin-resistant*Staphylococcus aureus* and vancomycin-resistant enterococci.	Hydrofiber absorbent dressings used for exudative wounds.
Debriding agents	Hypergel (hypertonic saline gel), Santyl (collagenase), Accuzyme (papain urea)	Various products provide some chemical or enzymatic debridement.	Debriding agents are useful for necrotic wounds as an adjunct to surgical debridement.
Foam	LYOfoam, Spyrosorb, Allevyn	Polyurethane foam has absorptive capacity.	These dressings are useful for cleaning granulating wounds with minimal exudate.

Table 2.2 Continued.

Hydrocolloid	CombiDERM, Comfeel, DuoDerm CGF Extra Thin, Granuflex, Tegasorb	Hydrocolloid dressings are made of microgranular suspension of natural or synthetic polymers, such as gelatin or pectin, in an adhesive matrix. The granules change from a semihydrated	Hydrocolloid dressings are useful for dry necrotic wounds, wounds with minimal exudate and for clean granulating wounds.

		state to a gel as the wound exudate is absorbed.	
Hydrogel	Aquasorb, DuoDerm, Intrasite Gel, Granugel, Normlgel, Nu-Gel, Purilon Gel, KY Jelly	Hydrogel dressings are water-based or glycerin-based semipermeable hydrophilic polymers; cooling properties may decrease wound pain. These gels can lose or absorb water depending upon the state of hydration of the wound. They are secured with secondary covering.	These dressings are useful for dry, sloughy, necrotic wounds (eschar).
Low-adherence dressing	Mepore, Skintact, Release	Low-adherence dressings are made of various materials designed to remove easily without damaging underlying skin.	These dressings are useful for acute minor wounds, such as skin tears, or as a final dressing for chronic wounds that have nearly healed.
Transparent film	OpSite, Skintact, Release, Tegaderm, Bioclusive	Transparent films are highly conformable acrylic adhesive films with no absorptive capacity and little hydrating ability. They may be vapor permeable or perforated.	These dressings are useful for clean, dry wounds with minimal exudate. They also are used to secure an underlying absorptive material, to protect high-friction areas and areas that are difficult to bandage (eg, heels) and to secure intravenous catheters.

If we take a simple laceration or cut inflammation begins after injury and the wound site swells as the biochemical ingredients needed for wound healing gather: leukocytes and monocytes fibrinogen, histamine, prostaglandins, and vasoactive substances. A great deal happens during this stage – it must occur to prepare the wound for the succeeding phases of wound healing. In fact, conventional drugs that limit inflammation such as non-steroidal anti-inflammatories (NSAID's) will slow the healing of a wound! Next, fibroblasts begin to proliferate and position themselves for collagen synthesis. As collagen content increases, the

wound site strengthens. The third and final stage of wound healing lasts the longest. This maturation, or remodeling phase, may continue for weeks or several years (depending on the severity of the injury), with gradual improvements in wound appearance. After operations or surgical procedures, the body may take time to adjust and achieve the harmonious balance it had before and gradual wound healing should be supported. Take your time to recover and follow your chosen medical professional's advice.

Many herbal and homeopathic remedies have been formulated with specific ingredients to promote wound healing and support the body's ability to resist infection. Herbs such as Agrimonia eupatoria (a well-known astringent which helps to tighten and constrict tissues) and Achillea millefolium (named after Achilles, the Greek mythical figure, who used it to stop the bleeding wounds of his soldiers) can help to heal wounds naturally. Calendula officianalis is an effective first-aid solution for all minor burns and scalds as well as any skin abrasion or cuts Calendula will also benefit the wound healing process from the inside out by stimulating the natural process of healthy cells and tissue regeneration. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences (Myers et al, 1980). Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally (Mather et al, 1989), or when it is suppressed by various agents like corticosteroids (Ehrlich & Hunt, 1968), anti-neoplastics (Raju & Kulkarni 1986), or non-steroidal anti-inflammatory agents. Medical treatment of wound includes administration of drugs

either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (Savanth & Shah, 1998). The topical agents used include antibiotics and antiseptics (Chulani, 1996), desloughing agents (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment) (Savanth & Mehta, 1996), wound healing promoters (e.g. Tretinoin, aloe vera extract, honey, comfrey, benzoyl peroxide, chamomilia extract, dexpanthenol, tetrachlordecaxide solution, clostebol acetate and the experimental cytokines. Various growth factors like platelet derived growth factor, macrophage derived growth factor, monocyte derived growth factor (Mather et al, 1989) etc. are necessary for the initiation and promotion of wound healing. Many substances like tissue extracts (Udupa et al, 1991), vitamins & minerals and a number of plant products (Dahanukar et al, 2000) have been reported by various workers, to possess pro-healing effects. Wound healing herbals encourage blood clotting, fight infection and accelerate the healing of wounds. Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction a number of herbal products are being investigated at present. Various herbal products have been used in management and treatment of wounds over the years.

2.3 Pomegranate (Punica Granutm)

2.3.1 Pomegranate Plant History

According to legend, pomegranates grew in the Garden of Eden, and the fruit has been used as a folk medicine for thousands of years. More recently, it has been promoted as a "superfood" that can relieve symptoms of many diseases. In laboratory tests, pomegranate shows